$O_2Br$ case, the strength of the O–Br bond is expected to be less than that of O–Cl, which results in the observed *increase* in the $\nu_1$ frequency (1441 → 1487 $cm^{-1}$). Interestingly enough, this trend in the $\nu_1$ frequencies of the $O_2X$ species (X = H, F, Cl, Br) parallels the reverse trend in the bond dissociation energies of the O–X radical molecules (Table II). It would thus appear that factors other than electronegativity must be included in any bonding scheme involving the $X-O_2$ or related X–NO moities. Furthermore, in any such bonding scheme, the rather unique properties of fluorine must be taken into consideration.

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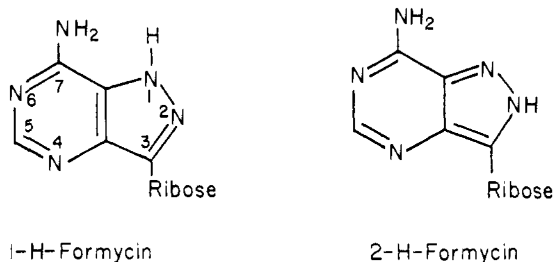
### Dynamics of Tautomerization of Formycin

*Sir:*

Both experimental and theoretical investigations have shown that formycin A (7-amino-3β-D-ribofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine), an important analogue of adenosine, undergoes a temperature-dependent tautomeric equilibrium between N(1) H and N(2) H forms.[1-5] Some of these




studies suggest that the N(1) H tautomer is dominant, although under some conditions the N(2) H form can be detected in amounts of 15% or so.[5] Above the formycin absorption maximum of 295 nm, the two tautomers have different extinction coefficients, with the N(2) H tautomer having the greater absorbance.[1,6] This situation makes it possible to study the temperature-dependent tautomerization by absorbance measurements. In addition, it opens the possibility for exploring the dynamics of tautomerization by temperature-jump relaxation methods, with an optical detection system.

We found that, although in buffered solutions (e.g., 2 mM imidazole, pH 7) the absorbance changes are too rapid ($\tau < 10^{-5}$ s) to follow by our temperature-jump system,[7] in unbuffered solutions (e.g., 0.1 M NaCl, pH 5–7) a single relaxation process is easily detected with a time constant of $\sim 10^{-4}$ s at 25 °C. The relaxation time is concentration independent over the range of $10^{-5}$ to $10^{-3}$ M. This verifies that a unimolecular process is responsible for the relaxation. However, the relaxation time $\tau$ shows a significant pH dependence that is most easily described by eq 1 where F and F′ are formycin

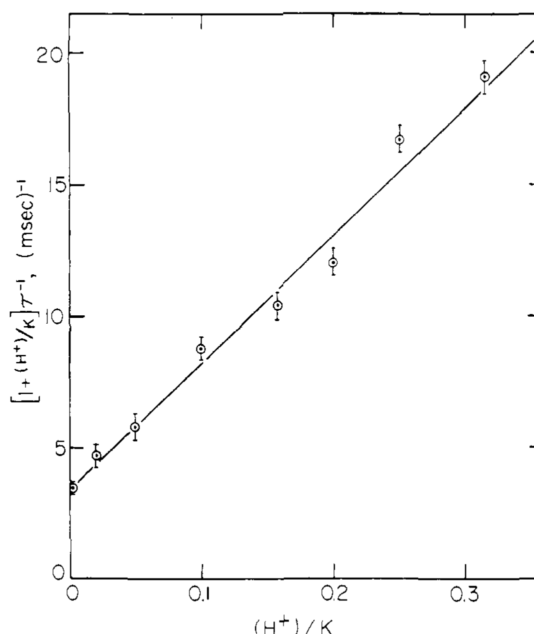
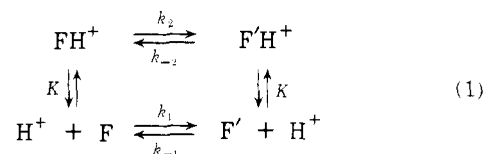



**Figure 1.** Plot of $[1 + (H^+)/K](1/\tau)$ vs. $(H^+)/K$, for 0.1 M NaCl, 25 °C. Individual points are averages over a range of formycin concentration of 10 μM to 1 mM. The line was determined by the method of least squares. Error brackets correspond to the range of observed values.



$$\begin{array}{ccc} FH^+ & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & F'H^+ \\ K\updownarrow & & \updownarrow K \\ H^+ + F & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & F' + H^+ \end{array} \quad (1)$$

tautomers. Assuming the vertical ionization steps are rapidly equilibrated (and have roughly similar p$K$ values), the relaxation time for this mechanism is simply given by

$$[1 + (H^+)/K](1/\tau) = (k_1 + k_{-1}) + [(H^+)/K](k_2 + k_{-2}) \quad (2)$$

According to eq 2, a plot of $[1 + (H^+)/K]\tau^{-1}$ vs. $(H^+)/K$ should yield a straight line with slope of $(k_2 + k_{-2})$ and an intercept of $(k_1 + k_{-1})$.

Figure 1 gives a plot of the data, according to eq 2. It is clear that a strictly linear relationship is obtained between $[1 + (H^+)/K](1/\tau)$ and $(H^+)/K$, where the spectrophotometrically determined (by us) value of $K = 10^{-4.5}$ was used. (This p$K$ value (for N(6)) agrees well with other independent determinations.[8,9]) From this plot we obtain (at 25 °C) $k_1 + k_{-1} = 3.4 \times 10^3\ s^{-1}$ and $k_2 + k_{-2} = 4.9 \times 10^4\ s^{-1}$. (Errors in values of $k_1 + k_{-1}$ and $k_2 + k_{-2}$ are estimated as ±15%.) Thus, with formycin in the protonated form, tautomerization is much faster (also, see below). As far as we know, this is the first determination of tautomerization rate constants at 25 °C for these kind of compounds.

We assumed that the microscopic p$K$'s for the two vertical ionization steps are identical. The data fit well with the simple assumption. Also, the data do not give a good fit if the single p$K$ is varied beyond the range of p$K$ = 4.5 ± 0.2. If the two microscopic p$K$'s are close in value, then there should be no uptake or release of protons accompanying the horizontal tautomerization steps. This expectation was confirmed by finding that the relaxation is not observed when one attempts to follow it by transient changes in a pH indicator. Since the two vertical ionization steps have about the same equilibrium

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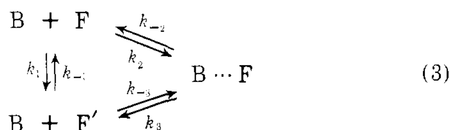
constants, detailed balancing requires that $k_1/k_{-1} \approx k_2/k_{-2}$.

The relaxation time is substantially shortened by the presence of basic species such as imidazole, citrate, glycine, and acetate. The shortening is a linear function of the amount of unprotonated basic species added. A simple scheme that explains the data is given in eq 3, where B stands for base and



B···F is a transient complex present in vanishingly small concentrations. By applying the steady-state assumption to (B···F), we obtain for the reciprocal relaxation time $\tau_b^{-1}$ in the presence of base

$$\tau_b^{-1} = k_{ss}(B) + (k_1 + k_{-1}) \quad (4)$$

where the steady-state constant $k_{ss}$ is given by

$$k_{ss} = \frac{k_2k_3 + k_{-2}k_{-3}}{k_{-2} + k_3} \quad (5)$$

For imidazole (B = unprotonated form) we obtain $k_{ss} \approx 10^8$ $M^{-1}\,s^{-1}$.

Since the rate is shortened in a linear fashion with increasing (B), the rate-determining step in tautomerization is undoubtedly the abstraction by B of a proton from N(1) (or N(2)); the resulting transient intermediate then quickly adds back a proton from the solvent or species BH. In the absence of buffer species such as imidazole, the faster tautomerization of the protonated form ($k_2 + k_{-2} \gg k_1 + k_{-1}$; see eq 1 and 2 ff) presumably occurs because the positive charge helps to stabilize the transient anion generated in the pyrazole part of the ring during the rate-determining step.

The pyrazole ring nitrogen has a pK of ~9.5.[9] For a proton-transfer reaction in which $pK_{acceptor} < pK_{donor}$, the rate of transfer is reduced below that of the diffusion controlled rate (because the acceptor binds the proton more weakly than the donor).[10,11] Consistent with this expectation is the value of $\sim 10^8\ M^{-1}\,s^{-1}$ for $k_{ss}$ for imidazole, a value which is below the diffusion controlled limit.[10]

The rapid rate ($1/\tau > 10^3\ s^{-1}$) of formycin tautomerization demonstrated here is faster than the turnover rates for many enzymes. This means that, even though the 1-H tautomer is predominate, the quick tautomerization to the 2-H form occurs sufficiently fast so as not to limit the rate of an enzymatic process that is specific for the 2-H species.

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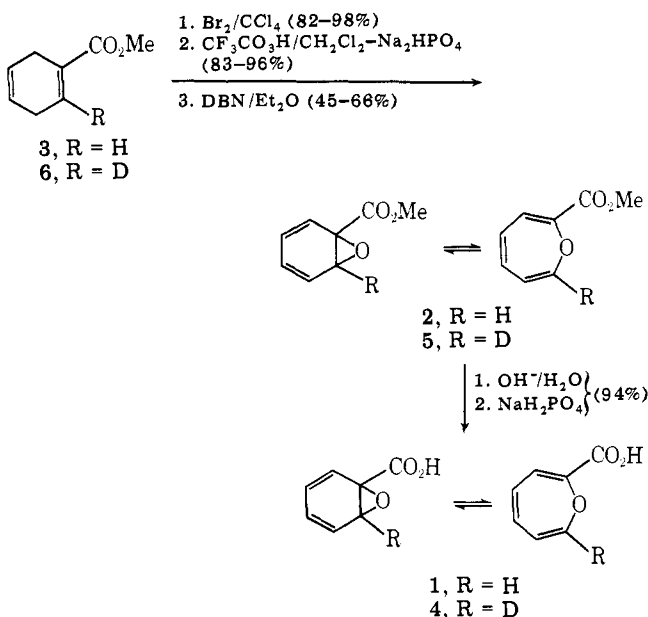
## Synthesis and Aromatization of 2-Carboxy- and 2-Carbomethoxyoxepin–Benzene Oxide

*Sir:*

The metabolism of aromatic substrates in mammals, microorganisms, and higher plants by initial formation of arene oxides and subsequent aromatization to phenols via the NIH shift pathway is well documented.[1] Many of the monooxygenase-catalyzed ortho hydroxylations of substituted benzenes[1a,b,f,g,2] probably proceed by such a pathway involving the arene 1,2-oxide, but, with the exception of 2-methyloxepin–benzene oxides,[1a,b,d,e] few 2-substituted arene oxides have been available for aromatization studies to establish whether the fate of substituents is consistent with the observed biological reaction.

In various studies 2-carboxyoxepin–benzene oxide (**1**) and some of its derivatives have been postulated as biological intermediates in ortho hydroxylation or oxidative decarboxylation reactions. Salicylic acid biosynthesis from benzoic acid (or cinnamic acid via benzoic acid) in *Gaultheria procumbens* occurs with no migration of the carboxyl group and with migration and retention (16–35%) of ortho tritium labeling.[2b] The latter observation, while of lower retention of tritium than expected, is nevertheless consistent with a significant contribution by the NIH shift pathway, and presumably would involve the 1,2- or 2,3-oxide of benzoic acid. Haslam has suggested an unusual biosynthesis of salicylic acid from **1**.[3] The 1,6-oxide of salicylic acid has been suggested as an intermediate in the salicylate hydroxylase catalyzed oxidative decarboxylation to catechol,[4] and similar arene oxide intermediates may be involved in the biological oxidative decarboxylations of substrates such as phenazine-1-carboxylic acid[5] and *p*-aminobenzoic acid.[1h,6]

In view of the interest in **1** as a biosynthetic intermediate, we have prepared **1** by hydrolysis of methyl ester **2** and investigated the aromatization reactions of **1** and **2**.[7] Bromination of **3**[8] at the $C_4$–$C_5$ double bond, epoxidation at the $C_1$–$C_2$ double bond, and dehydrobromination afforded **2** as stable yellow liquid. Hydrolysis of **2** in aqueous base, acidification with $NaH_2PO_4$, and rapid extraction gave pure **1** as yellow crystals (mp 68–72 °C dec). Although prepared in a state of high purity, **1** suffers decomposition to a mixture of phenol and salicylic acid in the crystalline state or in solution over a period of several hours. Phenol formation is favored with increasing pH (salicylic acid:phenol[9]) as follows: pure $CF_3CO_2H$ (64:36),

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